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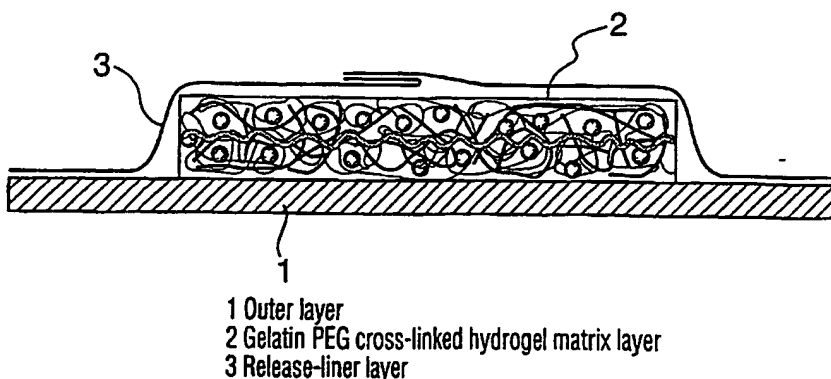
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(54) Title: **HYDROGEL WOUND DRESSING CONTAINING LIPOSOME-ENCAPSULATED THERAPEUTIC AGENT**



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(57) Abstract: The present invention is directed to a hydrogel wound dressing which effectively releases a therapeutic agent over a prolonged period of time. Liposomal therapeutic agents, such as antibiotics and antiseptics, are incorporated into hydrogels and the hydrogel/liposomal matrix is formed into a sheet. This hydrogel/liposomal sheet is placed on top of an outer layer (optionally coated with a medical-grade adhesive) and then applied to a wound. The present invention is particularly useful for treating and preventing infections in wounds.

## HYDROGEL WOUND DRESSING CONTAINING LIPOSOME-ENCAPSULATED THERAPEUTIC AGENT

### TECHNICAL FIELD

The present invention is directed to a multi-layer wound dressing that releases an effective amount of a therapeutic agent over a prolonged period of time. The multi-layer wound dressing of the present invention comprises: an outer layer; a cross-linked hydrogel matrix layer, with an optional support material confined within the hydrogel matrix; a liposome-encapsulated therapeutic agent incorporated in the hydrogel layer; and, optionally, a protective release sheet. The present invention further provides means for treating or alleviating cutaneous wound infections. The present invention also provides a method of manufacture of the wound dressing product.

### BACKGROUND ART

Dressings to be applied to various types of wounds, including burns, surgical incisions and inflicted wounds, ideally promote healing, provide protection against infection, and prevent pooling of wound exudate. Furthermore, the dressings should be as comfortable as possible and should not cause or contribute to ancillary problems, such as bed sores. Moreover, the dressings should be translucent so that the wound may be easily visualized and monitored.

Products currently available for wound dressings include hydrogels. Hydrogels are complex formulations of hydrophilic cross-linked polymers (e.g., polyethylene oxide, polyacrylamides, and polyvinylpyrrolidone). Despite their high water content (up to 96%), hydrogels can absorb a slight to moderate amount of wound exudate, thereby preventing pooling of wound exudate. They are particularly useful as dressings for many partial-thickness skin defects (e.g., shallow abrasions, superficial wounds), blisters, decubitus ulcers, second-degree burns, and healthy, granulating tissue. Hydrogels are available as amorphous gels, as well as in a wafer or sheet form. The hydrogel sheets may include an adhesive border or be secured by applying a secondary dressing (e.g., stretch gauze, tubular bandage, and large film dressing). Examples of commercially available hydrogel sheets include: Geliperm™ (Geistlich-

Pharma/Fougera), Geliperm™ (Geistlich-Pharma/Fougera), Vigilon™ (Bard), Bard Absorption Dressing™ (Bard), Cutinova Gelfilm™ (Biersdorf), Elasto-gel™ (Southwest Technologies), AQUASORB™ (DeRoyal), CarraDres™ (Carrington Laboratories Inc.), 2<sup>nd</sup> Skin™ (Spenco Medical Ltd), Derma-Gel™ (Medline Industries), FLEXDERM™ (Dow Hickman Pharmaceuticals Inc.), AcryDerm™ (AcryMed), THINSite Transorbent™ (B. Braun), ClearSite™ (Conmed Corporation), CURAGEL™ (Kendall) and NU-GEL™ (Johnson & Johnson).

10 A variety of patents have described the use of liposomes within a hydrogel. For example, U.S. Patent No. 5,064,655 describes a liposomal gel composition suitable for topical application. U.S. Patent No. 5,094,819 is directed to fluorophore-containing liposomes immobilized in a hydrogel. U.S. Patent No. 5,843,647 discloses a stent coated with a hydrogel which optionally comprises liposomes containing DNA. U.S. Patent No. 5,879,713 is directed to enhanced delivery of biologically active molecules using a hydrogel to immobilize the bioactive molecules at the site of release. U.S. Patent No. 5,942,245 discloses liposomes having SOD enzymes encapsulated therein, wherein the liposomes are incorporated into carriers such as hydrogels. U.S. Patent No. 6,048,546 discloses a method of preparing a lipid-bilayer material, such as polymerized liposomes, wherein the lipid-bilayer material is  
20 encapsulated in a gel. U.S. Patent No. 4,897,269 describes a pharmaceutical composition containing liposomes, which encapsulate an active compound, dispersed in a gel for topical application. However, none of these referenced patents disclose or suggest a wound dressing which comprises a gel comprising liposomes.

Topical application of a hydrogel comprising liposomes (without a protective outer layer) results in the gel rapidly drying up, and thus long-term release of the drug is not obtained. There is a need in the art for a liposomal hydrogel wound dressing that does not dry out and will release a therapeutic agent at a controlled rate over an extended period of time.

30 The patent literature is replete with references to various hydrogel wound dressings. See, for instance, U.S. Patent Nos. 5,489,262 and 5,106,629 (transparent hydrogel wound dressing); and U.S. Patent No. 4,909,244 (a hydrogel wound dressing adapted for preventing pooling of wound exudate). A distinctive disadvantage of the

currently available hydrogel wound dressings is that they do not provide a barrier against wound infection. In attempts to overcome this problem, commercially available bactericidal dressings contain charcoal that is activated by the wound exudate (Actisorb™ and Actisorb Plus™, Johnson & Johnson; Odor Absorbent Dressing™, Hollister; Lyofoam C™, Seton Healthcare Ltd.). Recently, silver-coated antimicrobial barrier dressings were developed for the treatment of burn wounds, graft and donor sites, chronic wounds (including pressure ulcers, diabetic ulcers, etc.), and post-surgical wounds (Acticoat™, Westaim Biomedical Corp.; Aerglaes™, Maersk). Several wound dressings are also impregnated with antibiotics or antibacterial agents. For example, some gauze dressings are impregnated with chlorhexidine (Bactigras™, Smith & Nephew; SEROTULLE™, Leo Laboratories Ltd.; Clorhexitulle™, Hoescht), framecycin (Fucidin-Intertulle™, Leo Laboratories Ltd; Sofra-Tulle™, Hoechst-Roussel), bismuth tribromophenate (Xeroform™, Chesebrough-Pond's), scarlet red (Scarlet Red Dressing™, Chesebrough-Pond's), or povidone-iodine (Inadine™, Johnson & Johnson; Povidern™, Seton Healthcare).

While those medicated paraffin gauzes are recommended as low adherent wound dressings, they have a tendency to dry. Unless those dressings are changed very frequently, they become incorporated into the newly formed granulation tissue, thus causing undesirable damage to a healing wound site upon their removal. Furthermore, some studies have shown that the repeated use of these medicated gauzes was associated with contact dermatitis and the emergence of antibiotic-resistant strains of micro-organisms.

Presently, there are no commercially available hydrogel wound dressing sheets comprising within the hydrogel matrix a therapeutic agent. However, it is often recommended clinically that an antimicrobial agent be applied under the hydrogel dressing or blended with the amorphous hydrogel. While this method ensures some control of bacterial growth, it is not always practical as it introduces another step in the wound care management. Therapeutic substances have been added to gel pads or bandages to provide additional bacterial control and other therapeutic effects. Examples of medicated hydrogel products are disclosed in U.S. Patent No. 5,753,257 (burn dressing), U.S. Patent No. 5,260,066 (cryogel bandage containing therapeutic agent), U.S. Patent No. 5,695,777 (absorptive wound dressing for wound healing

promotion), U.S. Patent No. 4,552,138 (dressing material based on a hydrogel, and a process for its production), and in W.O. Patent No. 9820916A1 (antimicrobial-coated hydrogel-forming absorbent polymers).

10 Wachol-Drewek *et al*, *Biomaterials* 17:1733-1738 (1996) disclose the use of collagen implants of various structures and a gelatin sponge which were placed in antibiotic solutions and allowed to absorb the compounds. They concluded: "If an implant that has a protective effect against wound infections over a period of 24-48 h is required, the materials described here are suitable. However, where treatment in infected areas should ensure antibiotic cover for 5-10 d[days] neither collagen materials immersed in antibiotics nor collagen sponges containing gentamicin are suitable."

20 The therapeutic agents contained in the aforementioned wound dressings are not immobilized within the hydrogel matrix. Those skilled in the art will appreciate that in the presence of wound exudate, the drug is thus rapidly released at the injured site, so that a long-lasting local therapeutic effect cannot be achieved. In recent years, liposomes have been increasingly explored as novel drug delivery systems that alleviate this problem.

30 Liposomes are microscopic spheres that are produced when a phospholipid thin film is hydrated under particular conditions. Conditions of high shear, produced during extrusion of the lipid solution through 100 nm pore size membranes, typically yield liposomes of 100 nm diameter. Liposomes have been used as drug delivery systems for many years, because they are biocompatible; non-immunogenic; non-toxic; allow encapsulation of water soluble and hydrophobic drugs equally well; allow solubilization of recalcitrant drugs; reduce toxicity of certain drugs; and, allow a slow, continuous release of their contents over a relatively long period of time. Typically, liposomes can release their drug content over a 7-14 day period, or even longer if required. Liposomes can be custom-designed by varying the lipid type, lipid and cholesterol ratio, the surface charge, surface charge density, size and mode of production. Careful attention must be given to the formulation and drug encapsulation efficiency of liposomes in order to produce a slow-release drug reservoir. In essence, liposomes are an excellent drug delivery vehicle.

Furthermore, one study described the use of a topical liposomal povidone-iodine hydrogel combined with moisturizer for antiseptic treatment of wounds [Reimer, K., *et al.*, *Povidone-iodine liposomes—an overview*. *Dermatology*, 1997. **195**(Suppl 2): p. 93-9]. U.S. Pat. 5,863,556 issued to Rückert *et al.* disclosed liposomal pharmaceutical preparations incorporating an active agent (e.g., antiseptic, anesthetic, or wound-healing agent) that could be added to gels, ointments and lotions.

As discussed above, the advantage of a hydrogel wound dressing is that it allows for hydration of the wound site, while simultaneously removing excess moisture and providing for a barrier from outside contaminants. However, the prior art has not disclosed hydrogel wound dressing sheets that deliver, in a uniform and controlled manner, a therapeutically effective amount of one or more therapeutic agents entrapped in a plurality of liposomes dispersed throughout the hydrogel. The advantage of having a therapeutic agent delivered to the wound site is that the therapeutic agent can further promote wound healing and prevent infection. Thus, there is a need for a hydrogel wound dressing which effectively keeps a wound hydrated, while simultaneously removing excess moisture from the wound, wherein the hydrogel wound dressing releases a therapeutic agent to the wound site over a period of time to promote wound healing and prevent infection.

## DISCLOSURE OF THE INVENTION

The invention provides a therapeutic wound dressing which comprises an outer layer; a cross-linked hydrogel matrix layer; an optional hydrogel-supporting material confined within the hydrogel matrix; a liposome-encapsulated therapeutic agent incorporated in the hydrogel matrix; and an optional protective release sheet. The hydrogel matrix material is preferably cross-linked with polyethylene glycol or carbodiimide, and is positioned under the outer layer for placement on the wound.

The cross-linked hydrogel matrix is biocompatible and biodegradable (i.e. does not release potentially toxic degradation products), and will ensure protection of the liposomes from membrane-disrupting shear forces that are encountered during handling of the wound dressing, and from rapid degradation of the liposome *in vivo*. By adjusting the amount of cross-linking within the hydrogel matrix, it is possible to

control the rate of release of the therapeutic agent. For example, with higher levels of cross-linking, one gets slower release of the therapeutic agent. With reduced cross-linking, one gets a quicker release of the therapeutic agent.

The containment of the liposomes within the hydrogel matrix creates an opportunity to control drug diffusion rates. One may vary the type of liposome employed to optimize drug release and distribution profiles. Therefore, by adjusting the amount of cross-linking and the type of liposome used, the applicants found that they could optimize the rate of release, thereby affording release of the drug over several days.

The liposomes used in the present invention may be unilamellar or multilamellar. Liposomes, microspheres, nanospheres, biodegradable polymers, and other systems are excellent drug delivery vehicles, and the methods of preparation and drug loading procedures for liposomes and the others are well known in the art (see, for example, U.S. Patent Application Serial No. 08/843,342 by DiCosmo et al). Liposomes can store both polar and non-polar compounds via interactions with the biocompatible and biodegradable lipid bilayer, or compartmentation within the aqueous core, respectively.

The liposomal hydrogel of the present invention includes a variety of gel drug combinations. Generally, the selection or pairing of the hydrogel and drug is determined only by the desired application and relevant indication. That is, any active agent that can be compounded into liposomes, microspheres, nanospheres, or other suitable encapsulation vehicle can be confined within the hydrogel matrices of the present invention, which are then used to create the wound dressings of the present invention.

The hydrogel matrix thus constitutes a vehicle for the containment of high concentrations of therapeutic agent such as one or more antibiotics, antiseptic agents, hormones, steroids, growth factors, antihistamines, colony stimulating factors, interleukins, and the like, and/or combinations thereof. The therapeutic hydrogels of the present invention can be used in wound dressings for the management of wound infection and to promote wound healing. The liposomes, which are incorporated

within the hydrogel and encapsulate the therapeutic agent, act as controlled drug delivery depots by releasing the drug over several days. The hydrogel wound dressings of the present invention are applied to a wound and the drug is then released by diffusion from the hydrogel to the wound site onto which the hydrogel wound dressing is applied.

10 The hydrogel matrix can be a conventional hydrogel (e.g., gelatin, pectin, etc.), a protein (e.g. collagen, etc.), or other adjuvant. The hydrogel matrix of the present invention is cross-linked and preferably will have some structural support to impart resistance to shear forces. As discussed above, by adjusting the amount of cross-linking within the hydrogel matrix, one can effectively control the rate of drug diffusion. A preferred hydrogel is gelatin cross-linked with polyethylene glycol as by reacting gelatin with nitrophenylcarbonate- or disuccinimidylcarbonate-PEG. Another preferred hydrogel is gelatin cross-linked with carbodiimide.

20 The therapeutic hydrogels of the present invention serve as support material for a variety of liposomal therapeutics. Any therapeutic agent suitable for encapsulation in a liposome, microsphere, nanosphere or the like can be utilized in the present invention. For example, therapeutic agents useful in the present invention include antibiotics, antiseptic agents, antihistamines, hormones, steroids, therapeutic proteins, and the like.

It will be appreciated by those of ordinary skill in the art that the desired concentration of therapeutic agent within a hydrogel will vary depending upon the characteristics of the chosen therapeutic agent. For example, as between an antibiotic and a therapeutic protein, the required concentration of antibiotic, which are generally active in the microgram range, will likely be higher than the concentration of a therapeutic protein, many of which are active in the nanogram range.

30 The present invention provides a multi-layer wound dressing comprising: an outer layer; a cross-linked hydrogel matrix layer, with an optional support material confined within the hydrogel matrix; at least one liposome-encapsulated therapeutic agent incorporated in the cross-linked hydrogel matrix; and optionally a protective release sheet.

The present invention further provides a multi-layer wound dressing which provides for the long term, stable release of a therapeutically effective amount of at least one therapeutic agent, wherein said wound dressing comprises: an outer layer, a cross-linked hydrogel matrix layer, and a therapeutic agent encapsulated in liposomes and incorporated in said hydrogel matrix layer.

10 By long term, stable release is meant the release of a stable therapeutic agent over a period of at least about 24 hours, and preferably up to about 14 days. By therapeutically effective amount of a therapeutic agent it is meant an amount suitable to achieve the desired therapeutic effect, depending on the agent employed. For example, a therapeutically effective amount of an antibiotic is that which is capable of reducing the bacterial counts in the wound significantly below the threshold considered to be a clinical infection (i.e.,  $10^5$  colony forming units per g of tissue). It will be appreciated that the therapeutically effective amount of the therapeutic agent will vary depending on the agent employed and the conditions requiring treatment. One of ordinary skill in the art relying on established medical principles will be able to readily determine the appropriate agent and the therapeutically effective amount required.

20 The present invention also provides a multi-layer wound dressing which provides for the effective distribution of a therapeutic agent, wherein said wound dressing comprises: an outer layer, a cross-linked hydrogel matrix layer, and a therapeutic agent encapsulated in liposomes and incorporated in said hydrogel matrix layer.

The present invention also provides methods for formulating such a multi-layer wound dressing. The present invention also provides means for treating or alleviating cutaneous wound infections. The type of therapeutic agent encapsulated in the liposome is not restricted to any single therapeutic agent. Therapeutic agents which  
30 can be used in the present invention include, but are not limited to, antibiotics, hormones, growth factors and other factors that are beneficial for the condition under management, in accordance with sound medical judgment.

The present invention also provides a method of treating wounds comprising applying a wound dressing of the present invention to a wound.

It is furthermore an object of the present invention to provide a method for protecting wounds from infection, comprising applying a wound dressing of the present invention to a wound.

10 A preferred embodiment of the present invention is a wound dressing comprised of a gelatin hydrogel cross-linked with polyethylene glycol (PEG), wherein dispersed within the hydrogel is a liposomal antibiotic or a liposomal antiseptic.

#### Definitions:

By hydrogel or gel it is meant any material forming, to various degrees, a jelly like product when suspended in a solvent, typically water or polar solvents. These gels can be proteins such as collagen or hemoglobin, or more conventional hydrogels such as gelatin, pectin, and fractions and derivatives thereof.

20 By liposomal therapeutic agents it is meant any physical structure surrounding or encapsulating a therapeutic agent such as a drug. Thus, liposomal therapeutic agents will include various drugs or biologically active agents such as antibiotics, antiseptic agents, antihistamines, hormones, steroids, growth factors, colony stimulating factors, interleukins, and the like confined or encapsulated within a structure such as a liposome, whether of unilamellar or bilayer structure, or microspheres or nanospheres or the like.

#### BRIEF DESCRIPTION OF THE DRAWINGS

30 Figure 1. A cross-sectional view of the preferred wound dressing of the present invention illustrating the three layers that form the wound dressing.

Figure 2. An enlarged view of the hydrogel matrix layer (2) illustrated in Figure 1.

Figure 3. A cross-sectional view illustrating the two layers that form a second preferred embodiment of the hydrogel wound dressing product.

Figure 4. A bar graph representing the effectiveness of liposomal ciprofloxacin-loaded hydrogel wound dressings in treating a full-thickness wound infection in rats. Wounds were covered with either a plain liposome hydrogel wound dressing (black bars) or a liposomal ciprofloxacin hydrogel wound dressing (dashed bars). Number of animals in each experimental group is indicated in parenthesis. Data are means  $\pm$  SEM.

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Figure 5. A bar graph representing the effectiveness of liposomal ciprofloxacin-loaded hydrogel wound dressings in preventing the progression of infection in contaminated full-thickness wounds in rats. Wounds were covered with either a plain liposome hydrogel wound dressing (black bars) or a liposomal ciprofloxacin hydrogel wound dressing (dashed bars). Number of animals in each experimental group is indicated in parenthesis. Data are means  $\pm$  SEM.

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Figure 6. A bar graph comparing the bactericidal efficacies of liposomal ciprofloxacin-loaded hydrogel wound dressings and commercially available silver-coated barrier wound dressings in a rat model of contaminated full-thickness burn wounds. Wounds were excised after 30 min (dashed bars) or 120 min (black bars). Number of wounds excised in each experimental group is indicated in parenthesis. Data are means  $\pm$  SEM.

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Figure 7. A bar graph representing the effectiveness of liposomal ciprofloxacin-loaded hydrogel wound dressings in treating an infection in a superficial muscle in rats. Wounds were covered with a plain liposome hydrogel wound dressing (black bars), a free ciprofloxacin hydrogel wound dressing (empty bars) or a liposomal ciprofloxacin hydrogel wound dressing (dashed bars). Number of animals in each experimental group is indicated in parenthesis. Data are means  $\pm$  SEM.

Figure 8. A bar graph representing the effectiveness of liposomal ciprofloxacin-loaded hydrogel wound dressings in treating an infection in a deep muscle in rats. Wounds were covered with a plain liposome hydrogel wound dressing

(black bars), a free ciprofloxacin hydrogel wound dressing (empty bars) or a liposomal ciprofloxacin hydrogel wound dressing (dashed bars). Number of animals in each experimental group is indicated in parenthesis. Data are means  $\pm$  SEM.

## MODES FOR CARRYING OUT THE INVENTION

10 A major object of the present invention is to provide a therapeutic wound dressing, characterized in that the wound dressing comprises an outer layer; a cross-linked hydrogel matrix layer; an optional gel-supporting material confined within the hydrogel matrix; a liposome-encapsulated therapeutic agent incorporated in the hydrogel matrix; and an optional protective release sheet. The hydrogel matrix of the present invention can be hydrogel (e.g. gelatin, pectin, etc.), a protein (e.g. collagen, hemoglobin, etc.), or other adjuvant. The wound dressing of the present invention is optionally translucent. Furthermore, the outer layer of the wound dressing optionally is coated with a medical-grade adhesive, to facilitate binding of the hydrogel matrix to the outer layer. Additionally, the outer layer may optionally be perforated, vapor permeable, and/or waterproof.

20 A further object of the present invention is a method of treating a wound by applying the hydrogel wound dressing of the present invention. The hydrogel wound dressing of the present invention provides an improved level of wound management for a wide range of applications, including burn wounds and full thickness wounds.

30 It is possible to modulate the rate of release of the therapeutic agent. For instance, by increasing the amount of crosslinking, one can slow down the rate of release of the therapeutic agent. Conversely, by decreasing the amount of crosslinking, one can speed up the rate of release of the therapeutic agent. Another means of controlling the release of the therapeutic agent is by selection of the appropriate liposome. Different therapeutic agents have different properties, and one of skill in the art would know how to match particular therapeutic agents with particular liposomes to arrive at desired properties, such as rate of release of a therapeutic agent. Thus, by adjusting the amount of crosslinking in the hydrogel matrix and the type of liposome used, one can effectively control the rate of release of the therapeutic agent.

Furthermore, by providing an outer layer to the top of the hydrogel matrix, the inventors discovered that one can extend the period of time the therapeutic agent is released. This is because without the outer layer, the hydrogel matrix quickly dried up and once this occurred, the liposomes no longer released the therapeutic agent. The outer layer used in the present invention prevents the hydrogel matrix from drying up (while simultaneously releasing excess moisture from the hydrogel matrix) thereby allowing for extended release of the therapeutic agent.

10 In one preferred embodiment of the present invention, depicted by Figure 2, a gelatin hydrogel matrix (b), cross-linked with polyethylene glycol or carbodiimide (c) in which a drug encapsulated by a liposome (d) is dispersed, is made to form a thin, flexible sheet by procedures known in the art and disclosed herein. As shown in Figure 1, the hydrogel sheet (2) is positioned in the center portion of a larger diameter adhesive outer layer (1). The thin-film outer layer (1) is preferably selected from a group of materials including, but not limited to, polyurethane, polyethylene, vinyl, polyvinylchloride, or other suitable material. The perimeter portion of the outer layer (1) is coated with a medical-grade adhesive, and is preferably perforated to allow the skin of the patient to breath. Those skilled in the art will appreciate that this outer layer (1) is required to maintain an adequate hydration level of the hydrogel matrix, as  
20 well as to prevent maceration of the wound by allowing release of excess moisture. The outer layer (1) and hydrogel sheet (2) optionally are translucent and optionally the outer layer (1) has a grid pattern transposed thereon to allow for easy measurement and viewing of the wound. The medicated hydrogel sheet can be manufactured to any shape and size.

In another preferred embodiment of the invention (Figure 1), two overlapping release sheets (3) are provided. The release sheets can be made of, for example, non-adherent, plasticized material. Each release sheet (3) is attached to the adhesive perimeter portion of the outer layer (1), and extends towards the center of the dressing to cover half of the hydrogel matrix (2). The free edge of one of the release sheets (3) is folded at the center of the dressing, while the free edge of the other release sheet (3) lies flat on top of the folded edge. As the edges of the release sheets (3) are peeled back, the hydrogel wound matrix (2) located in the center of the dressing is placed in contact with the wound, followed by the adhesive portion of the  
30

outer layer (1). Those skilled in the art will appreciate that this release system preserves sterility of the wound dressing by minimizing contact with the hydrogel matrix (2) *per se*. The ready-to-use wound dressings can then be sealed preferably in pouches that are impermeable to water vapor, and sterilized (e.g., by  $\gamma$ -irradiation).

The drug incorporated in the liposomes (Figure 2, d) is preferably selected from a group of drugs including, but not limited to antibiotics, antimicrobials, antipathogenic peptides, antiseptic, antibacterial and antifungal agents, anti-inflammatory drugs, local anesthetics, central nervous system acting agents, and wound-healing promoting agents (e.g., growth factors). The choice of the method for loading the drug into the liposomes is dictated by the chemical properties of the target compound. Those skilled in the art will appreciate that the concentration of the drug entrapped in liposomes is a function of the intrinsic activity of the therapeutic agent. Furthermore, those skilled in the art will also appreciate that the composition (e.g., lipid type, lipid and cholesterol ratio, surface charge, etc.) of the liposomes can be customized depending on the drug encapsulated to produce an appropriate slow-release drug reservoir. Typically, polyethylene glycol cross-linked gels containing dipalmitoylphosphatidylcholine/ dipalmitoylphosphatidylethanolamine- polyethylene glycol/cholesterol or dipalmitoylphosphatidylcholine/cholesterol liposomes constitute the optimum formulation with respect to gel matrix stability, liposome efflux, and drug loading efficiency.

Those skilled in the art will also appreciate that a wound dressing that includes an adhesive border is not necessarily suitable for covering very large (e.g., entire torso or back) or irregular surface areas. Thus, in a second preferred embodiment of the invention (Figure 3), a proprietary hydrogel matrix (2) that incorporates a liposomal drug is made to form a thin, flexible sheet by pouring a fluid hydrogel mixture into an appropriate mold. An adhesive outer layer (1) is then applied to the hydrogel matrix sheet (2) to fit its dimensions. In this second preferred embodiment, the thin-film outer layer (1) is preferably selected from a group of materials including, but not limited to polyurethane, polyethylene, vinyl, polyvinylchloride, or other suitable material. In this second preferred embodiment of the invention, the hydrogel wound dressing sheet is secured to the wound using secondary dressings (e.g., bandages, tubular dressings).

Those skilled in the art will also appreciate that a large hydrogel sheet may require inclusion of a support material to facilitate its handling and reduce risks of tear of the hydrogel matrix. Thus, in the second preferred embodiment of the invention (Figure 3), an optional support material (3) is included in the dressing to confer strength to the hydrogel matrix layer (2). The permeable nature of the support material allows the hydrogel matrix to completely impregnate the material, so that the hydrogel matrix is entirely exposed at the outer edges of the support material. In this way, the support material does not adhere to the wound. This is highly desirable since wound healing is impaired if a dressing adheres to the newly formed granulating tissue. The support material may be selected from the group of materials consisting of strands of natural fibers, strands of synthetic fibers, knitted fabrics, woven medical-grade fabrics or meshes, non-woven medical-grade fabrics or meshes, or any combination thereof.

The following examples show by way of illustration, and not by way of limitation, the practice of the present invention. The invention is in no way limited to the type of hydrogels or liposomes used in the following examples, nor to the method of forming the cross-linked hydrogels of the present invention. As stated previously, the type of therapeutic agent incorporated within the liposomes will determine what liposome will be used, what hydrogel will be used, and what method of formulating the hydrogel wound dressings of the present invention will be used. These examples present data showing the effectiveness of the liposomal ciprofloxacin-loaded hydrogel wound dressing either in treating established full-thickness wound infections (Example 1), or in preventing the progression of infection in contaminated wounds (Example 2).

Those skilled in the art will appreciate that although ciprofloxacin is the only antimicrobial agent exemplified, many other liposomal drugs (e.g., antibiotics, antiseptic, growth factors, as well as antimicrobial and antifungal agents) may also be successfully incorporated in the gel wound dressing sheet to exert therapeutic effects.

## EXAMPLE 1

## LIPOSOME AND PEG-GELATIN GEL PREPARATION

Liposomes that are incorporated into the hydrogels may be composed of a variety of lipids. Typically, dipalmitoyl phosphatidyl choline (DPPC)/cholesterol (1:1) is used. For example, applicants prepared liposomes composed of DPPC/Cholesterol/PEG-DSPE/Rhodamine-DPPE in a 1:1:0.05:0.001 ratio. The formulation to be used is not limiting, and any number of lipid-to- other-constituents ratios may be used to effectively achieve the embodiments of this invention. The usual procedure for liposome synthesis begins by dissolving the resulting lipid in a small volume of chloroform (i.e. 4 mL) followed by solvent removal *in vacuo* for approximately 2h. The resulting lipid film is hydrated at 45°C with an appropriate volume of 300 mM ammonium sulfate for ciprofloxacin-loaded liposomes, or a mixture of 2% chlorhexidine digluconate and 150 mM glucose ofr chlorhexidine-loaded liposomes. Liposomes are then frozen in liquid nitrogen and thawed in a 45°C water bath (5X), followed by high-pressure extrusion through two 100 nm-pore membranes (10X). This procedure has been shown to produce unilamellar liposomes with an average diameter of 100 nm and an equal solute distribution between the exterior and interior of the liposomal membrane. M. J. Hope, M. B. Bally, G. Webb, and P. R. Cullis, "Production of large unilamellar vesicles by a rapid extrusion procedure. Characterization of size distribution, trapped volume and ability to maintain a membrane potential," *Biochim. Biophys. Acta*, **812**:55-65 (1985); L. D. Mayer, M. J. Hope, P. R. Cullis, and A. S. Janoff, "Solute distributions and trapping efficiencies observed in freeze-thawed multilamellar vesicles," *Biochim. Biophys. Acta*, **817**:193-196 (1986). Chlorhexidine liposome suspensions are diluted five-fold before removal of non-encapsulated drug. For ciprofloxacin-loaded liposomes, external ammonium sulfate is removed by overnight dialysis against 1000 volumes of 10 mM MES buffer and 150 mM NaCl (pH 4.5); unencapsulated chlorhexidine is removed by ultracentrifugation at 100,000 x g for 1h with resuspension in MES/saline (3X). External ammonium sulfate can also be removed by passing the suspension through a G-50 column (1 X 10 cm) and eluting with a 10% sucrose solution (pH 4.0).

Therapeutic agent was incorporated in the liposomes according to the remote-loading technique described in Y.K. Oh et al, "Formulation and efficacy of liposome-

encapsulated antibiotics for therapy of intracellular *Mycobacterium avium* infection," *Antimicrob. Agents Chemother.* 39:2104-2111 (1995).

After removal of the external, unloaded drug, chlorhexidine-containing liposomes can be immediately mixed with PEG and gelatin to initiate hydrogel formation. Ammonium sulfate liposomes can be loaded with ciprofloxacin after incorporation within the hydrogel matrix, by incubation of the liposome-hydrogel in a solution of antibiotic, typically 2-10 mg/mL, for 1 hr at 50°C.

10           The hydrogel matrix when cross-linked with PEG (PEGXL) consists of 10% gelatin, 6%NPC-PEG and 10% sucrose at pH4.0, and self cross-linked hydrogel (SelfXL) contains 10% w/v gelatin. Both formulations of hydrogels can be prepared by dissolving the components in a suspension of 100 nm liposomes at 45°C for approximately 15 min. The hydrogels are kept at 4°C for at least 10 min prior to cross-link initiation, that involves immersion of the gels into a 200 mM, pH 9, borate buffer solution. However, self cross-linked gels must first be activated by reaction with water soluble carbodiimide (5 mg/mL) for 1 h. Cross-linking excipients are removed from the gels by continual washing with buffer for 24 h. If liposomes were required, they were added from a pure liposome suspension. The concentration of liposomes in  
20           PEG-gelatin solutions was 15 mM with respect to DPPC. All solutions were heated at 45°C for 15 min. to dissolve gelatin.

#### FORMULATION SELECTION

The selection of a satisfactory liposomal hydrogel formulation is based upon the optimal combination of three major criteria:

- (1) Stability of cross-linked gelatin gels under physiological conditions (+/- liposomes);
- (2) Liposome drug loading efficiency and release kinetics; and
- 30           (3) Liposome affinity for gelatin-based gels.

An initial study was undertaken to determine the quantity of activated PEG required to achieve a cross-linking extent sufficient to ensure stability of the gel matrix at 37°C. Gels with varying levels of activated PEG were placed in buffered saline (pH

7.4) maintained at 37°C. At regular intervals, the medium's absorbance at 280 nm was monitored as an indication of gel degradation. A starting ratio of 1:1 or 1.5:1 of activated PEG termini to gelatin amino groups was necessary to maintain gel integrity for extended periods at 37°C (10 to 15% loss of starting material after 30 days). Due to price/performance considerations, 1:1 PEG-gelatin gels were used in all subsequent studies. Gels cross-linked with carbodiimide were even more stable with only an approximate 5% loss of starting material after 30 days at 37°C. The stability of liposome-containing PEG cross-linked (PEGXL) and self cross-linked (SelfXL) gelatin gels was also determined by 37°C incubation in PBS or 50% calf serum for 7 days. The dry weights of the cross-linked lipogels at the end of the experiment were not significantly different than their initial dry weights indicated that either cross-linking method is compatible with the presence of liposomes in the gel matrix. Tensile strength tests also verified that liposome inclusion does not compromise the integrity of cross-linked gelatin matrices. Cross-linked PEG gelatin gels (with and without DPPC/cholesterol liposomes) could be stretched to approximately twice their original length before breaking, whereas non-cross-linked gels could only be extended to approximately 1 and 1/3 of their original length before rupture occurred.

Drugs may be incorporated in liposomes by either of two general drug-loading strategies, often referred to as active and passive loading. Weak amines, such as ciprofloxacin, can be actively loaded into pre-formed liposomes at high concentrations through the generation of a trans-bilayer pH gradient (acidic inside) that traps the protonated compound within the liposome core. Many other compounds, especially lipophilic ones, become associated with liposomes as they form, either through their co-formulation with lipids dissolved in organic solvent or by their inclusion in the medium used to hydrate the lipid film. The choice of loading strategy is usually dictated by the chemical properties of the target compound, and in the case of ciprofloxacin either strategy may be used. The ciprofloxacin encapsulation efficiencies of various liposome formulations as a function of lipid composition and drug loading method were measured. Egg PC liposomes containing 50 mole% cholesterol could retain significantly more drug than DPPC/cholesterol liposomes when the passive loading protocol was used (168 µg/umol phospholipid vs. 40 µg/umol). However, drug release from egg PC liposomes incubated in buffered saline at 37°C was more rapid relative to DPPC liposomes. Application of the active loading method with

respect to DPPC/cholesterol liposomes increased their encapsulation efficiency to approximately 180 µg/µmol phospholipid, which in combination with its slow release characteristics (<10% of initially encapsulated drug after 6 weeks at 4°C) suggested that the active loading of ciprofloxacin into DPPC/cholesterol liposomes provided the best compromise between maximizing drug loading and minimizing drug leakage.

10 The sequestering of DPPC/cholesterol liposomes in gelatin gels reduces the encapsulation efficiency of actively-loaded ciprofloxacin to approximately 100 µg/µmol phospholipid for reasons that are not clear. Interestingly, liposome formulations containing PEG lipid negate this effect, however, applicants did not use pegylated liposomes, thus avoiding any conflict with the pegylated STEALTH™ liposomes. The inclusion of charged lipids in the liposome formulation did not drastically alter the encapsulation efficiency of ciprofloxacin.

20 The affinity of neutral and pegylated liposomes (27 mM with respect to DPPQ for PEGXL and SelfXL gels was examined by monitoring liposome release from gels immersed in PBS (37°C) over a 7 day period. The highest initial efflux of liposomes (ca. 28%) was observed for pegylated liposomes in PEGXL gels, however, after 7 days all samples had released similar levels of their initial load of liposomes (ca. 26-34%).

The above data indicates that PEG cross-linked gels containing either DPPC/DPPE-PEG/cholesterol or DPPC/cholesterol liposomes constitute an optimum formulation with respect to gel matrix stability, liposome efflux, and drug loading efficiency.

#### RAT MODEL OF FULL-THICKNESS WOUNDS

30 On the experimental day, Sprague-Dawley rats were anesthetized and the dorsum was clipped, depilated, and cleansed using standard procedures. A custom-made plexiglass template with a one-cm<sup>2</sup> window was positioned over the depilated area. The location of the wound was then marked. The one-cm<sup>2</sup> piece of skin was surgically removed from the dorsal area of each animal, exposing the *panniculus carnosus* located above the *spinotrapezius* muscles.

After contamination of the wound, a hydrogel wound dressing was immediately applied to cover entirely the wound. Each wound dressing was then covered (see details in specific examples) to protect it from the animals. All animals were sacrificed 24 h after application of the dressing. The infected *panniculus carnosus* and part of the *spinotrapezius* muscles were dissected under aseptic conditions, and their bacterial content was assessed using standard microbiological procedures.

**BACTERIAL CHALLENGE.** A clinical isolate of *Pseudomonas aeruginosa* was used to infect the wounds. A bacterial suspension was obtained using standard microbiological procedures, and diluted to approximately  $1 \times 10^8$  colony forming units per mL. This bacterial dose was selected because the clinical definition of wound infection pertains to at least  $1 \times 10^5$  organisms/g tissue.

**HYDROGEL WOUND DRESSINGS.** Liposomal ciprofloxacin-loaded hydrogel wound dressings or plain liposome-loaded hydrogel wound dressings were supplied in individual vials (2-cm disks, 1.25 mm thick). Sterility of the hydrogel dressings of a given batch was assessed two days prior to their application on the wounds.

## EXAMPLE 2

This study was designed to assess the bactericidal efficacy of liposomal ciprofloxacin-loaded hydrogel wound dressings in treating established infections. Rats were anesthetized and a full-thickness wound was made, as previously described. Approximately  $5 \times 10^8$  colony forming units (in 500  $\mu$ L) of a clinical strain of *P. aeruginosa* was injected under the fascia of the *panniculus carnosus* muscle in each rat. A hydrogel dressing containing either plain liposomes or liposomal ciprofloxacin (2 mg ciprofloxacin) was immediately applied to cover entirely the wound. Each dressing was then covered with a Tegaderm™ (3M) semi-permeable membrane; a gauze pad was applied to ensure a good contact between the dressing and the wound; and a piece of tubular elastic dressing retainer as well as a rat tether jacket were fitted to each rat. All animals were sacrificed 24 h after application of the dressing and muscle tissues were excised and processed as previously described.

A 5-log reduction in bacterial counts measured in the *panniculus carnosus* muscle was observed after application of the liposomal ciprofloxacin hydrogel dressings for 24 h compared to the control dressings (Fig. 4). In contrast, there was a significantly lower reduction (half-log) in bacterial counts measured in the *spinotrapezius* muscles between these 2 experimental groups. These data demonstrate that application of a liposomal ciprofloxacin hydrogel wound dressing for 24 h significantly reduces bacterial counts in superficial tissues. However, this bactericidal effect is markedly reduced in deeper tissues. It is also noteworthy that several of the hydrogel wound dressings recovered after 24 h were dehydrated, to various extents. These data suggested that different types of backings were required to maintain the appropriate level of hydration of the hydrogel dressing, depending on the level of exudate from the wound. It is also noteworthy that at least 75% of the initial dose of ciprofloxacin was recovered in the hydrogel discs after 24h.

### EXAMPLE 3

This study was designed to simulate a scenario where a 'fresh' full-thickness wound was contaminated with bacteria. However, cleaning or debriding of the wound could not be performed immediately. Under those circumstances, first aid treatment consisted of applying an antibiotic-loaded wound dressing to attempt to limit the progression of the superficial infection to deeper tissues.

Rats were anesthetized and a full-thickness wound was made, as previously described. Approximately  $2 \times 10^8$  CFU (in 200  $\mu$ L) of a clinical strain of *P. aeruginosa* was deposited in the cavity created by removing the skin (i.e., directly on the *panniculus carnosus* muscle). A hydrogel dressing containing either plain liposomes (n=6) or liposomal ciprofloxacin (n=5; 2 mg ciprofloxacin) was applied to cover the entire wound. Each dressing was then covered with a small sterile piece of gauze; and an Elastoplast<sup>TM</sup> adhesive tape. A gauze pad was then applied to ensure a good contact between the dressing and the wound, and a piece of self-adherent elastic bandage was used to securely wrap the dressing around each rat. All animals were sacrificed 24 h after application of the dressing and muscle tissues were excised and processed as previously described.

All hydrogel wound dressings recovered were intact (i.e., no apparent dehydration). Assessment of bacterial counts in the *panniculus carnosus* and the *spinotrapezius* muscles revealed approximately 7-log and 3-log reductions, respectively, in the number of bacteria recovered in these tissues after application of the liposomal ciprofloxacin hydrogel dressings for 24 h compared to that of the control dressings (Fig. 5). These data suggest that application of liposomal ciprofloxacin hydrogel dressings for 24 h is an effective way for preventing the progression of infection in contaminated wounds. It is also noteworthy that at least 75% of the initial dose of ciprofloxacin was recovered in the hydrogel discs after 24 h.

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#### EXAMPLE 4

This study was designed to compare the bactericidal efficacy of liposomal ciprofloxacin-loaded hydrogel dressings to that of a commercially available silver-coated antibacterial barrier wound dressing. A modified Walker-Mason burn wound model was used. Briefly, four male, Sprague-Dawley rats were anesthetized. Their dorsum was shaved and cleansed using standard procedures. Six 2 cm x 2 cm burn wounds were made on the back of each animal by applying a heated brass rod for 40 s on the skin, to induce a full-thickness, non-lethal burn. Three 2 cm x 2 cm pieces of sterile gauze and three 2 cm x 2 cm silver-coated wound dressings were applied to cover the wounds in two rats. Each of the other two rats received a hydrogel wound dressing containing either plain liposomes (n=3) or liposomal ciprofloxacin (n=3; 2 mg ciprofloxacin). A mixture of *Ps. aeruginosa* Utah strain (approximately  $10^7$  CFU in 200  $\mu$ l) was then applied to each of the dressings. The animals were killed 30 min (n=2) or 120 min (n=2) after inoculation. The burn wounds were then excised under aseptic conditions. The wounds were sonicated separately, and bacterial counts were assessed using standard microbiological procedures.

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The reductions in bacterial count in the wounds 0.5 h and 2 h after application of the liposomal ciprofloxacin hydrogel and silver-coated wound dressings were comparable (Fig. 6). These data show that these wound dressings have comparable short-term bactericidal efficacies in burn wounds contaminated with Gram-negative organisms.

## EXAMPLE 5

This study was designed to determine the extent of bacterial contamination in a rat wound after a single application of a medicated hydrogel wound dressing for up to 7 days. Rats were anesthetized and a full-thickness wound was made, as previously described. Approximately  $5 \times 10^8$  colony forming units (in 500  $\mu$ L) of a clinical strain of *P. aeruginosa* was injected under the fascia of the *panniculus carnosus* muscle in each rat. A dressing containing plain liposomes, free ciprofloxacin (1 mg per disc), or liposomal ciprofloxacin (1 mg per disc) was immediately applied to cover entirely the wound. Each dressing was then covered with a small sterile piece of gauze, and an Elastoplast™ adhesive tape. A gauze pad was then applied to ensure a good contact between the dressing and the wound, and a piece of self-adherent elastic bandage was used to securely wrap the dressing around each rat. Animals were sacrificed after 1, 3 or 7 days following application of the experimental wound dressing. Muscle tissues were excised and processed as previously described.

Bacterial contamination in the *panniculus carnosus* and the *spinotrapezius* muscles remained constant in the control group throughout the 7-d study period. Bacterial counts were reduced by 70 % in the *panniculus carnosus* muscle 24 h after application of a hydrogel dressing containing free ciprofloxacin; these levels were not further affected by applying the dressing for up to 7 days (Fig. 7). In contrast, there was a gradual, 4-log reduction in the bacterial counts measured in the *panniculus carnosus* 7 days after application of a hydrogel dressing containing liposomal ciprofloxacin (Fig. 7). No reduction in bacterial counts measured in the *spinotrapezius* muscles was observed after application of a hydrogel wound dressing containing 1 mg of free or liposomal ciprofloxacin compared to the control dressings (Fig. 8). While application of the dressing containing free ciprofloxacin for 3 days reduced bacterial counts by 1-log, a 2.5-log reduction in bacterial counts was observed after application of the liposomal ciprofloxacin hydrogel dressings for the same period (Fig. 8). However, bacterial counts measured in the *spinotrapezius* muscles were comparable in the two drug-treated groups 7 days after application of the dressing (Fig. 8). These data demonstrate that application of a liposomal ciprofloxacin hydrogel wound dressing (1 mg ciprofloxacin) for up to 7 days significantly reduces bacterial counts in

superficial tissues compared to free drug. However, this enhanced bactericidal effect is not maintained in deeper tissues.

It will be apparent to those skilled in the art that the objects of the present invention, set forth above and made obvious in the preceding examples, are efficiently attainable. The examples described herein and the disclosure are intended to be illustrative and not exhaustive. These examples and descriptions will suggest many variations and alternatives to one of ordinary skill in the art. All of these alternatives and variations are conceivable within the scope of the patent claims.

**CLAIMS:**

1. A wound dressing, characterized in that said wound dressing comprises:
  - an outer layer;
  - a cross-linked hydrogel matrix layer; and
  - at least one therapeutic agent encapsulated in liposomes and incorporated in said hydrogel matrix layer.
- 10 2. The wound dressing of claim 1, characterized in that said cross-linked hydrogel matrix layer further comprises a support material selected from the group consisting of strands of natural fibers, strands of synthetic fibers, knitted fabrics, woven medical-grade fabrics, woven medical-grade meshes, non-woven medical-grade fabrics, non-woven medical-grade meshes, and combinations thereof.
3. The wound dressing of claim 1, characterized in that said cross-linked hydrogel matrix is selected from the group consisting of gelatin, pectin, and collagen.
- 20 4. The wound dressing of claim 1, characterized in that said cross-linked hydrogel matrix is gelatin cross-linked with polyethylene glycol.
5. The wound dressing of claim 1, characterized in that said cross-linked hydrogel matrix is gelatin self cross-linked.
6. The wound dressing of claim 1, characterized in that said cross-linked hydrogel matrix is gelatin cross-linked with carbodiimide.
7. The wound dressing of claim 1, characterized in that said outer layer is selected from the group consisting of polyurethane, polyethylene, vinyl and  
30 polyvinylchloride.
8. The wound dressing of claim 1, characterized in that said outer layer is coated with a medical-grade adhesive.

9. The wound dressing of claim 1, characterized in that said wound dressing is a translucent wound dressing.

10. The wound dressing of claim 9, characterized in that said outer layer has a grid transposed thereon.

11. The wound dressing of claim 1, characterized in that said outer layer is vapor permeable.

10 12. The wound dressing of claim 1, characterized in that said outer layer is perforated.

13. The wound dressing of claim 1, characterized in that said outer layer is waterproof.

14. The wound dressing of claim 1, characterized in that said wound dressing further comprises a protective release sheet.

20 15. The wound dressing of claim 1, characterized in that said therapeutic agent is selected from the group consisting of antibiotics, antimicrobials, antiathogenic peptides, antiseptics, antibacterial agents, antifungal agents, anti-inflammatory drugs, local anesthetics, central nervous system acting agents, wound healing promoting agents and combinations thereof.

16. The wound dressing of claim 15, characterized in that said antibiotic is a fluoroquinolone.

17. The wound dressing of claim 16, characterized in that said fluoroquinolone is ciprofloxacin.

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18. The wound dressing of claim 1, characterized in that said wound dressing comprises at least one therapeutic agent encapsulated in liposomes and incorporated in said hydrogel matrix layer.

19. The wound dressing of claim 1, characterized in that said hydrogel is composed of about 10% gelatin and about 6% NPC-PEG.

20. A wound dressing which provides for the long term, stable release of a therapeutically effective amount of an antibiotic agent, characterized in that said wound dressing comprises:

- an outer layer;
- a cross-linked hydrogel matrix layer; and
- a therapeutically effective amount of an antibiotic agent encapsulated in liposomes and incorporated in said hydrogel matrix layer.

21. The wound dressing of claim 20, characterized in that said antibiotic is a fluoroquinolone.

22. The wound dressing of claim 21, characterized in that said fluoroquinolone is ciprofloxacin.

23. A wound dressing, characterized in that said wound dressing comprises:

- a waterproof outer layer;
- a cross-linked hydrogel matrix layer comprising support material; and
- a therapeutic agent encapsulated in liposomes and incorporated in said hydrogel matrix layer.

24. A method for protecting wounds from infection comprising applying to the wound a wound dressing, characterized in that said wound dressing comprises:

- an outer layer;
- a cross-linked hydrogel matrix layer; and
- at least one therapeutic agent encapsulated in liposomes and incorporated in said cross-linked hydrogel matrix layer.

25. The method of claim 24, characterized in that said therapeutic agent is selected from the group consisting of an antibiotic, an antimicrobial, an antipathogenic

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peptide, an antiseptic, an antibacterial agent, an antifungal agent, and combinations thereof.

26. The method of claim 25, characterized in that said antibiotic is a fluoroquinolone.

27. The method of claim 26, characterized in that said fluoroquinolone is ciprofloxacin.

10 28. A method for protecting wounds from infection comprising applying to the wound a wound dressing which provides for the long term, stable release of a therapeutically effective amount of an antibiotic agent, characterized in that said wound dressing comprises:

an outer layer;

a cross-linked hydrogel matrix layer; and

a therapeutically effective amount of an antibiotic agent encapsulated in liposomes and incorporated in said cross-linked hydrogel matrix layer.

20 29. The method of claim 28, characterized in that said antibiotic is a fluoroquinolone.

30. The method of claim 29, characterized in that said fluoroquinolone is ciprofloxacin.

31. A method for treating wounds comprising applying to the wound a wound dressing, characterized in that said wound dressing comprises:

an outer layer;

a cross-linked hydrogel matrix layer; and

30 a therapeutic agent encapsulated in liposomes and incorporated in said cross-linked hydrogel matrix layer.

32. The method of claim 31, characterized in that said therapeutic agent is selected from the group consisting of antibiotics, antimicrobials, antipathogenic

peptides, antiseptics, antibacterial agents, antifungal agents, anti-inflammatory drugs, local anesthetics, central nervous system acting agents, and combinations thereof.

33. The method of claim 32, characterized in that said antibiotic is a fluoroquinolone.

34. The method of claim 33, characterized in that said fluoroquinolone is ciprofloxacin.

10 35. A method for treating wounds comprising applying to the wound a wound dressing which provides for the long term, stable release of a therapeutically effective amount of an antibiotic agent, characterized in that said wound dressing comprises:

an outer layer;

a cross-linked hydrogel matrix layer; and

a therapeutically effective amount of an antibiotic agent encapsulated in liposomes and incorporated in said cross-linked hydrogel matrix layer.

20 36. The method of claim 35, characterized in that said antibiotic is a fluoroquinolone.

37. The method of claim 36, characterized in that said fluoroquinolone is ciprofloxacin.

38. A method for preparing a wound dressing which comprises positioning a cross-linked hydrogel matrix sheet on a larger outer layer, characterized in that liposomes comprising therapeutic agents are incorporated within said cross-linked hydrogel matrix.

30 39. The method of claim 38, characterized in that said outer layer is coated with a medical-grade adhesive.

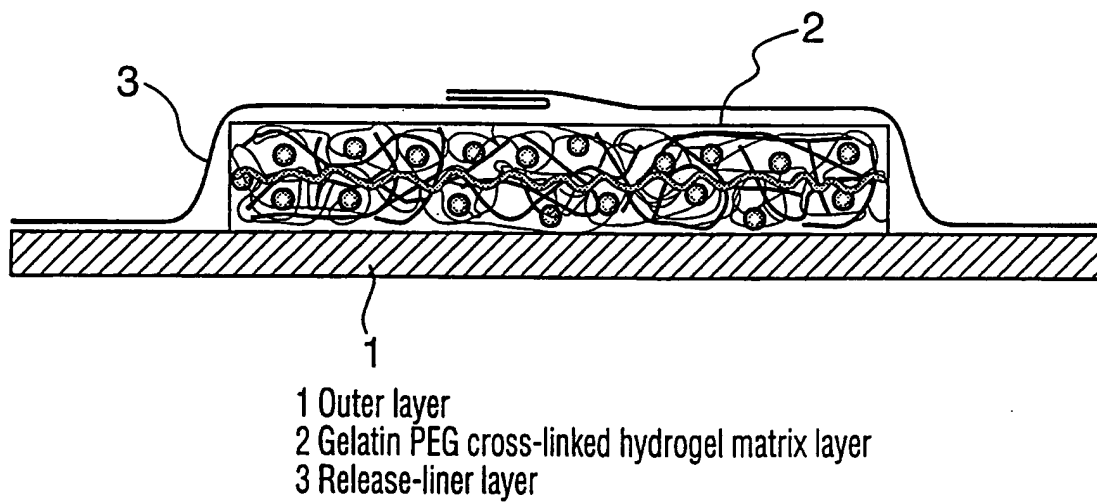
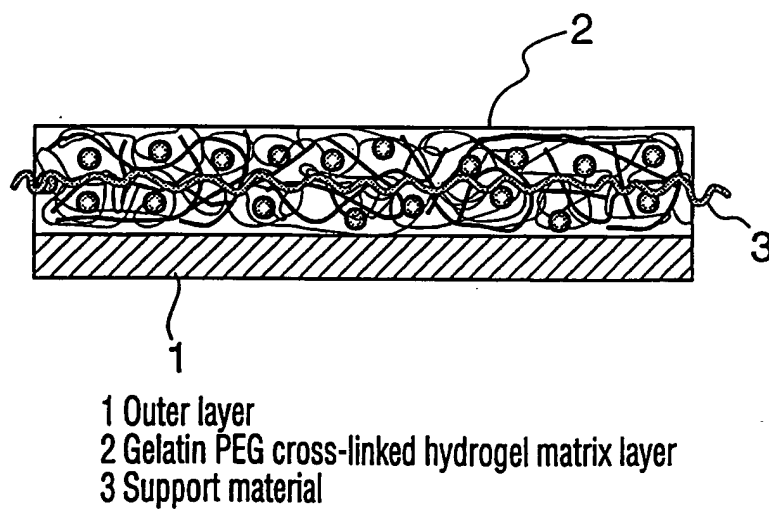
40. The method of claim 39, characterized in that said method further comprises:

attaching two release sheets to the adhesive perimeter on opposite sides of the cross-linked hydrogel matrix;

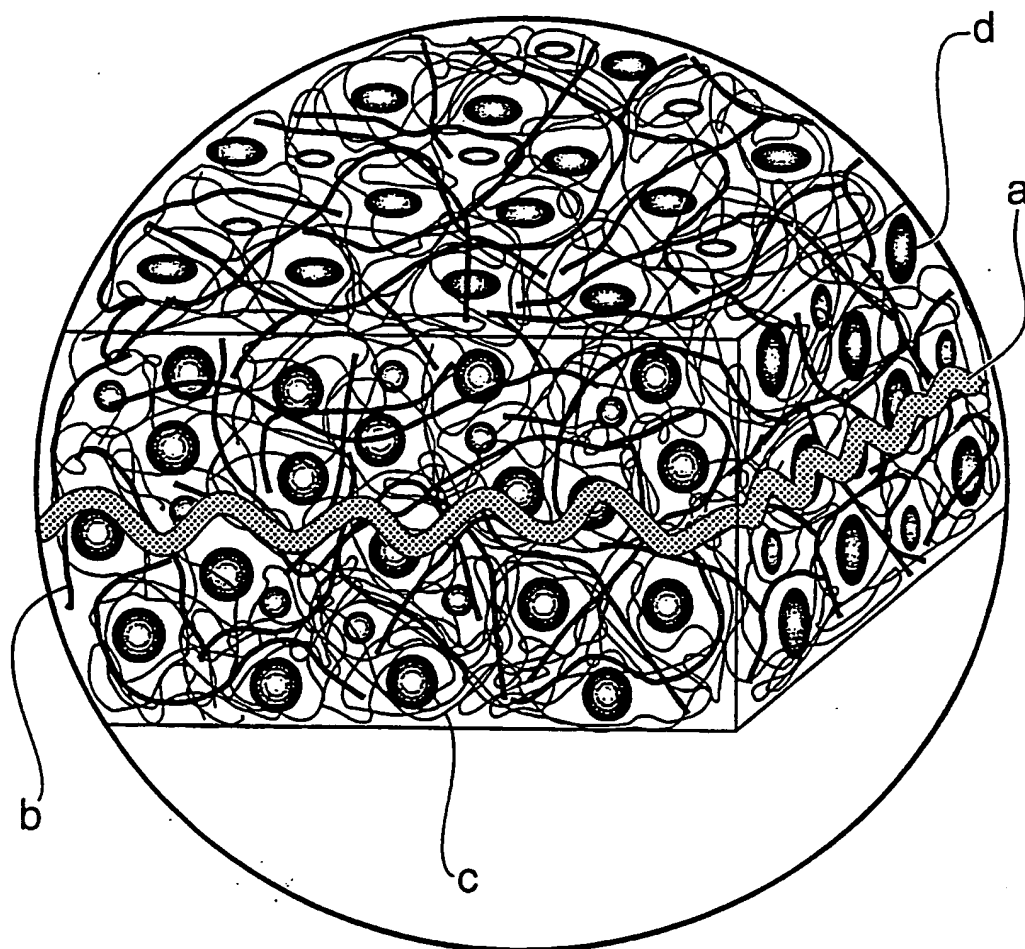
extending the release sheets towards the center of the dressing; and

folding the first release sheet at the center of the dressing, while allowing the free edge of the second release sheet to lie flat on top of the folded edge of said first release sheet.

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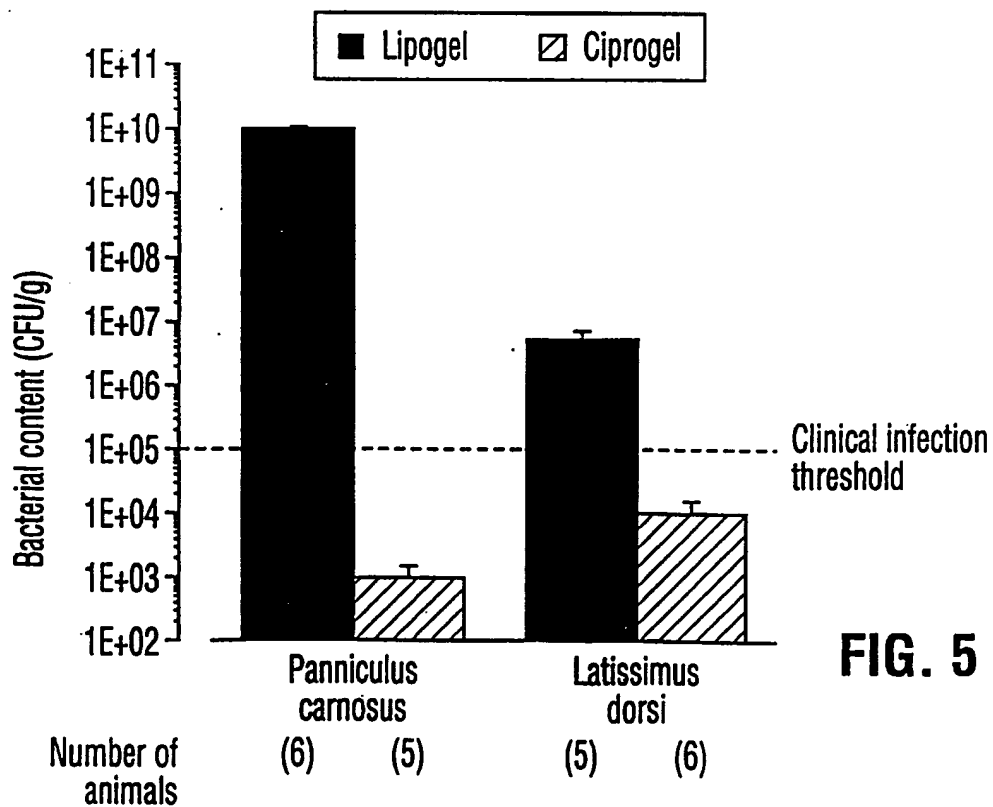
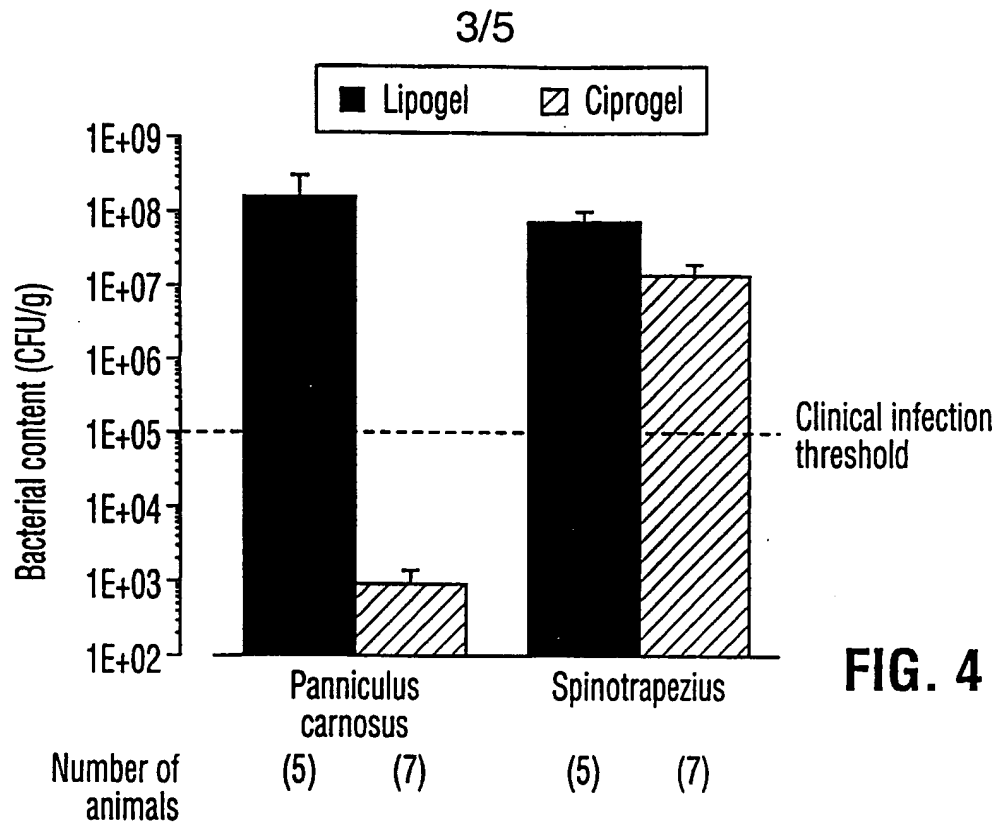
**FIG. 1****FIG. 3**

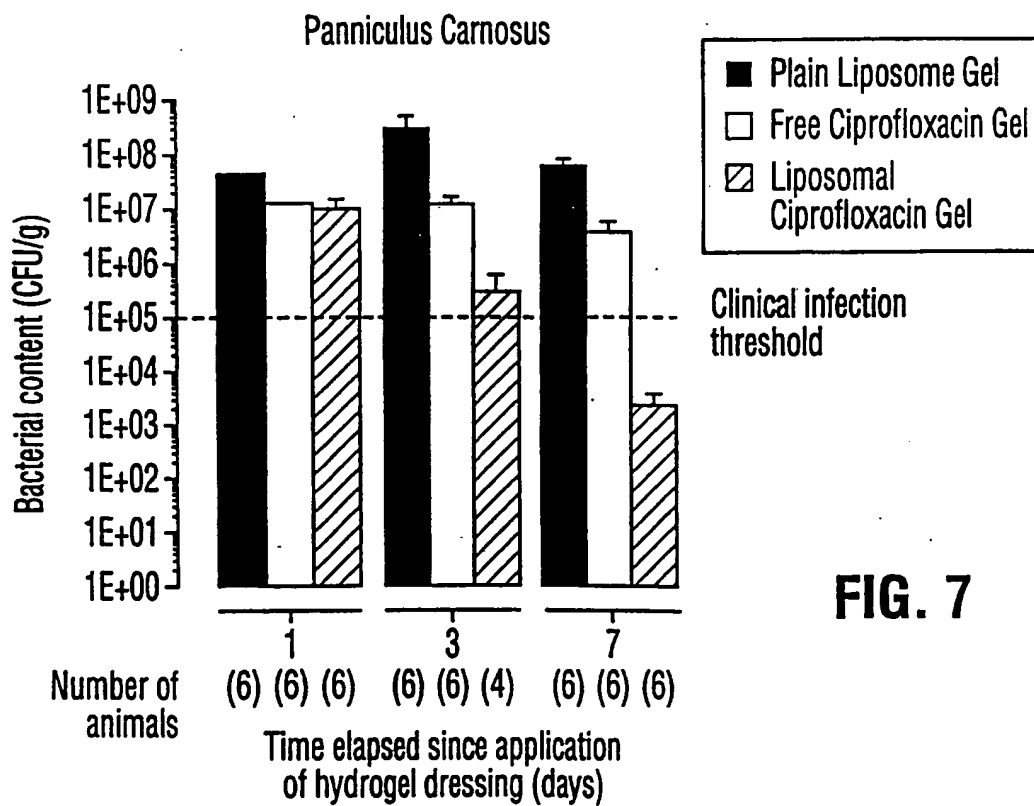
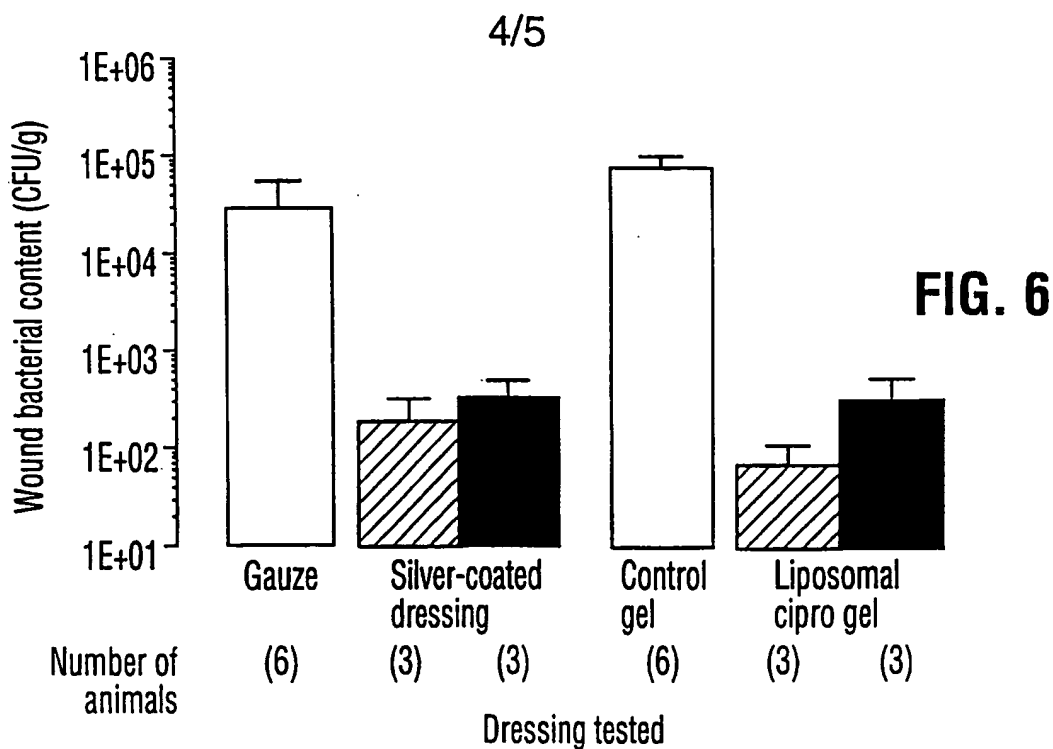
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a Support material  
b Gelatin  
c PEG cross-linker  
d Liposome

**FIG. 2**





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## Spinotrapezius

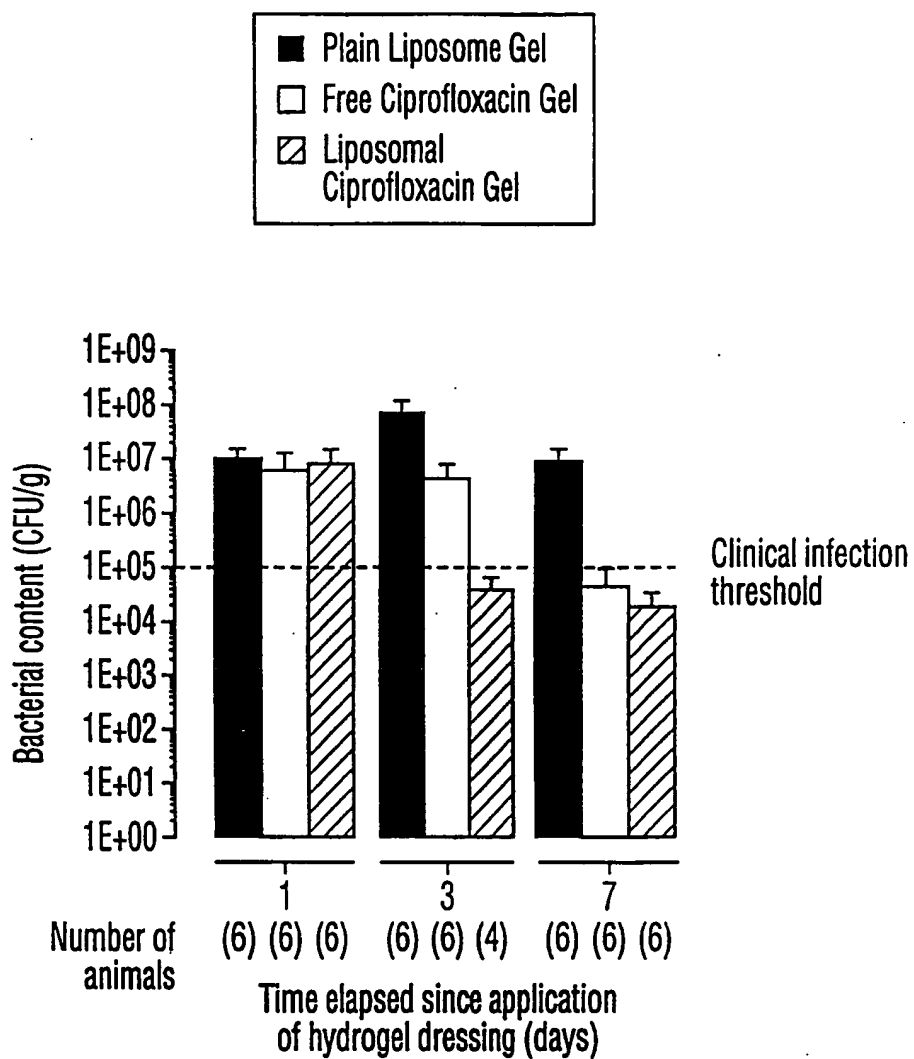


FIG. 8

## INTERNATIONAL SEARCH REPORT

Intern: al Application No

PCT/CA 00/00991

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 7 A61L15/44

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61L A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, FSTA, INSPEC, COMPENDEX

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5 718 914 A (FOLDVARI MARIANNA) 17 February 1998 (1998-02-17) column 2, line 23 - line 54 column 3, line 44 -column 4, line 14 figure 6 ---	1-40
Y	WO 98 46287 A (DICOSMO FRANK ;DITIZIO VALERIO (CA); UNIV TORONTO (CA)) 22 October 1998 (1998-10-22) page 14, line 22 -page 15, line 29 page 17, line 1 -page 18, line 22 page 21, line 15 -page 23, line 10 --- -/--	1-40

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents:

\*A\* document defining the general state of the art which is not considered to be of particular relevance

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\*O\* document referring to an oral disclosure, use, exhibition or other means

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\*Z\* document member of the same patent family

Date of the actual completion of the international search

8 December 2000

Date of mailing of the international search report

18/12/2000

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## INTERNATIONAL SEARCH REPORT

Intern: al Application No

PCT/CA 00/00991

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5 700 848 A (HEINTZ ROSWITHA A ET AL) 23 December 1997 (1997-12-23) abstract column 3, line 2 - line 52 column 4, line 16 - line 35 examples 5,15 -----	1-40
Y	WO 95 00155 A (VALIO BIOTUOTTEET OY ;LAATO MATTI KALEVI (FI); JALKANEN MARKKU TAP) 5 January 1995 (1995-01-05) page 2, line 28 -page 5, line 18 examples 2,3 figure 1B -----	1-40

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 24-37 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

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Continuation of Box I.1

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

# INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern: al Application No

PCT/CA 00/00991

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
US 5718914	A	17-02-1998	CA 2097163 A	02-12-1993
WO 9846287	A	22-10-1998	US 6132765 A	17-10-2000
			AU 7019898 A	11-11-1998
			EP 0984798 A	15-03-2000
US 5700848	A	23-12-1997	US 5837747 A	17-11-1998
			US 5846530 A	08-12-1998
			US 5705270 A	06-01-1998
			AU 3124793 A	07-06-1993
			CA 2121129 A	13-05-1993
			EP 0610441 A	17-08-1994
			JP 7503943 T	27-04-1995
			WO 9309176 A	13-05-1993
WO 9500155	A	05-01-1995	AU 6847894 A	17-01-1995
			EP 0711171 A	15-05-1996
			FI 956064 A	15-12-1995